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Role of Administration Routes of Anti - Infectious Bursal Disease Virus (Gumboro) Vaccine on Immunization of Chicken

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Abstract: The aim of the present work was to study the effect of the route of anti-IBDV vaccine administration on elevation of antibody titre and protective efficacy against Gumboro disease in addition the maternally derived antibodies titre (MDA) declining pattern was also studied. For this purpose 125 one day old progeny chicks from a known vaccinated dams were reared at an isolated pens and tested to determine anti IBDV antibody titre at day one (MDA) and day 17. At day 17 birds were divided into 5 groups; A , B, C and D based on the administration route of anti-IBDV vaccine while group E was not vaccinated and acted as negative control. Group A was vaccinated via intranasal (I/N) route. Group B was vaccinated via drinking water (oral) route. Group C was vaccinated via subcutaneous route. Group D was vaccinated via spraying (Inhalation) route. Each group was vaccinated twice at day 17 (Primary) and at day 24 (booster). Fifteen days post booster dose, sera samples were tested and birds were challenged to study the protective efficacy of the vaccine in each group. The mean antibody titre and coefficient of variation of group B were found to be superior to other groups. The protective efficacy parameters including mortality rate, gross lesions grading, bursa to body weight X 10^{-3} ratio and histopathologic changes grading were found to be better in group B than in other groups.

Key words: Ibd, vaccine, administration, immunization, Gumboro

Introduction

Diseases of chicken are mostly infectious in nature and therefore, a wide variability in losses due to such disease is expected. Singh *et al.* (1994) reported higher prevalence of infectious bursal disease at the age of 6-11 week more than in the age of 18-20 week. Infectious bursal disease is one of the most prevalent disease and inducing a morality of 40.4% (Anjum *et al.*, 1997).

Out of the infectious poultry diseases, infectious bursal disease stands-up as a major poultry disease in many countries (Zaheer *et al.*, 2003). The disease is now endemic in the most poultry producing areas of the world (Sharma *et al.*, 2000).

Infectious bursal disease and also known as Gumboro disease is acute highly contagious viral infection of young susceptible chicks (Lukert and Saif, 1997 and Hair-Bejo *et al.*, 2004). The disease by itself causes a degree of immunosuppression in affected birds (Zaheer *et al.*, 2003). The disease was first appeared in Delmarva area in 1958 and was reported by Cosgrov in 1962. It characterized by ruffed feathers, watery diarrhea, trembling and severe prostration (Ley *et al.*, 1983). It's characteristic gross lesions are in the lymphoid organs, primarily the bursa of Fabricious hence the name infectious bursal disease (IBD).

Effective vaccination against the disease, maintenance of healthy environment and good hygiene measures can reduce the incidence of the disease. The virus causing the disease has a potential for antigenic hetrogenicity which result in frequent outbreaks in the field even in flocks vaccinated against IBDV (Hassan *et al.*, 1998), still one of the significant component of the control of IBD is the vaccination which, if improved, may help in lowering the incidence of he disease in poultry.

All types of IBD vaccines used in Sudan are imported and comprise a variety of vaccine strains and applied through a variety of administration routes and different vaccination schedules for protection. In general many vaccination practices have been performed in poultry rearing field. However, despite the regular use of these vaccines and different vaccination practices the disease is still prevails in Sudan.

The present study was an effort to study the role of IBD vaccine administration route on the immunization of chicks using the degree of elevation of antibodies (ELISA) and protection against IBD efficacy as parameters.

Materials and Methods

Experimental chicks: One hundred and twenty five day-old broiler chicks were reared in 2x2 m^2 isolated pens for two weeks. When the MDA titre became low enough at day 17. The birds were divided into five groups; A, B, C, D and E of 25 birds each. Feed and water were given *ad libitum* and light was offered for 23 hours daily.

Route of vaccine administration and grouping of birds: Grouping of birds was based on the administration route

of IBD vaccine. Group A birds were vaccinated via intranasal (I/N) route. Group B birds were vaccinated via oral (drinking water) route. Group C birds were vaccinated via subcutaneous (S/C) route. Group D birds were vaccinated via spraying (Inhalation) route and group E birds were not vaccinated and acted as negative control.

All birds in group A, B, C and D were vaccinated with intermediate strain of infectious bursal disease (IBD) vaccine Nobilis D 78 (ID 4.5 ELD 50) twice at day 17 and day 24. The vaccine was obtained from Detasi Company, an agent of Intervet Company - Holland.

For all groups, 1000 dose IBD vaccine vial was first reconstituted in 50 ml distilled water. For group A (birds vaccinated via intranasal route 1.25 ml of the vaccine solution was used. Each bird in this group was vaccinated individually with 0.05 ml of the vaccine solution. For group B (vaccination via drinking water) birds were with held from water for two hours in the morning to insure that all birds will exposed to water containing anti-IBDV vaccine.1.25 ml of the vaccine solution was dissolved in 500 ml of distilled water that given to the birds. For group C 1.25 ml of the vaccine solution was diluted to a total of 5 ml with distilled water. 1 ml syringe was used for injecting each bird subcutaneously with 0.2 ml. For group D (birds vaccinated via spraying (inhalation) route 1.25 ml of the vaccine solution was diluted in 100 ml distilled water and used for spraying of birds after clumping together.

Serology (ELISA): Before vaccination blood samples were collected at day 1 from the heart and at day 17 (just before the primary dose of anti- IBD vaccination) and at day 39 (15 day post the booster dose of anti - IBD vaccination), from wing vein. Five birds from each group were bled using 1 ml syringe and kept overnight at room temperature (28 - 30°C) and serum was then separated in Eppendorff tubes and preserved at -20°C for IBD anti-bodies titre test.

Enzyme Linked Immunosorbent Assay (ELISA) Technique was performed as described by the manufacturer, ELISA reader and infectious bursal disease ELISA kit (Bio-Check company-Holland).

Sample to positive (S/P) ratio of the sample i.e. absorbance value of the tested serum divided by the absorbance of the positive control serum value was calculated to interpret the result. Coefficient of variation among individuals within each group was also calculated. Software programme was used to analyze the result as described by Blankfard and Silk (1989).

Challenge of the birds: For challenge of the birds, virus inoculum was prepared. Bursa of Fabricius samples were collected from IBD virus affected chicks from the field and were stored at 20°C till used. A 20%. (W/V) bursae homogenate was prepared by blending them in sterile phosphate buffered saline (PBS) (PH 7.4) along with antibiotic (Penicillin 1000 IU/ml, Streptomycin 10 mg/ml and Gentamycin 1 mg/ml). The preparation was freeze-thawed thrice and centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and filtered via sterile Whatman No 1 filter. Virus was tested against known positive antisera of IBDV by agar Gel precipitation test AG PT). The positive samples were stored at -20°C. this method was done as described by Khan *et al.* (1988) and Zaheer and Saeed (2003). For challenge, 1 ml of IBDV inoculum was injected via subcutaneous route at day 39.

Clinical signs: Clinical signs as described in OIE manual (2004) and by Ley *et al.,* (1983) were observed and classified as positive or negative.

Mortality rate: mortality rate in each group during the period post challenge till clinical signs of IBD disappeared was calculated as a ratio of the total number of birds at the day of challenge.

Postmortem gross lesions: postmortem gross lesions observed in every dead bird in each group were subjectively graded as normal (0), mild (1), mild to moderate (2), moderate (3) moderate to severe (4) and severe (5) based on the severity of the lesions on the bursa of Fabricius, body skeletal muscles surfaces, kidneys, erosions and hemorrhage of the mucosa of proventriculus (0IE manual 2004) and Ley *et al.* (1983).

Bursa to body weight ($\times 10^{-3}$) ratio: for bursa to body weight $\times 10^{-3}$ ratios bursa of Fabricius was removed and weighed for each dead bird in each group individually using a sensitive balance and then the average weight was calculated. The bursae were then preserved for histopathology. Average body weight of birds in each group were determined. The average bursa of Fabricius weight was then calculated as a ratio of average body weight multiplied by 10^{-3}.

Histopathology: For histopathology; bursa tissues were fixed in 10% buffered formalin. Each bursa was trimmed to the thickness of 5mm in size, fixed and dehydrated in a series of alcohol concentrations. Sectioning of tissues was done to a thickness of 5 micrometers in a microtome. The bursa tissues were mounted on glass slides and stained with haematoxyline and Eosin (H and E). Burae tissues were examined under microscope using X4, X10 and X40 objectives for histopathologic changes. Changes in bursa tissues were subjectively graded as normal (0), mild (1), mild to moderate (2), moderate (3), moderate to severe (4) and severe (5) according to Hair-Bejo *et al.* (2000) as a modified scoring for previously established method.

Table 1: Results of ELISA tested sera at day 1 and day 17 prior to experimental vaccination

Age	Mean titre	Min and max titre	N/S/P	S/P ratio	CV%
1 day	11222±1634.654	4860-13667	0/0/5	4.214±0.573	33 (OK)
17 days	291± 66.226	63-443	2/2/2001	0.512±3.153E-02	51 (high)

Table 2: Results of serologically (ELISA) tested sera of birds groups gathered according to the administration route of anti - IBD vaccine

Group	Mean titre	Min and Max titre	N/S/P	S/P ratio	CV%
A	459±289.1	663-1551	3/0/2	0.218±.129	141
B	5353±1384	2535-9018	0/0/5	2.126±.625	58
C	1124±524.84	378-3155	0/2/3	0.502±.217	121
D	216±60.09	63-361	5/0/0	0.118±2.596E-02	62
E	331±260.85	62-1374	4/0/1	0.160±.117	176

P = Positive, *Target titre = 2000-2500, S = Suspiecious, ** Positive cut off S/P = 0.20, N = Negative, *** S/P sample/absorbance of positive, **** CV = coefficient of variation, Group A: birds vaccinated via intranasal, Group B: birds vaccinated via drinking water, Group C: birds vaccinated via S/C injection, Group D: Birds vaccinated via spraying Group E: Non vaccinated birds (Control)

Table 3: Effeect of challenge of different groups of birds gathered according to the administration route of anti-IBD vaccine

Group	Gross lession grade	Clinical signs	Mortality	B/B.Wt×10^{-3} Ratio	Histopa.
A	2.98	Positive	8/25 (32%)	3.289±0.1608	2.25±0.12
B	2.67	Positive	3/25 (12%)	3.596±0.1704	2.41±0.21
C	3.28	Positive	5/25 (21%)	2.520±0.1979	3.12±0.52
D	3.21	Positive	7/25 (38%)	3.247±0.1335	3.25±0.24
E	3.63	Positive	8/25 (32%)	3.929±0.2573	3.62±0.13

Results and Discussion

One of the important questions addressed in this study was to determine the role of the route of administration of anti-IBDV vaccine on the immunization of chicks against Gumboro disease. Table 1 showed that MDA. At day one was fairly high and of almost equal levels and all samples was reacted positively, the mean titre using ELISA was 11222 ± 1635 and ranged from 4860 to 13667. The coefficient of variation (CV%) among chicks was 33% and considered as excellent and within the target CV% (20 - 50%). Sample absorbance to positive control absorbance ratio (S/P) results were fairly above the cut off point (0.20) among all tested sera with mean of 4.214 ± 0.573 and ranged from 1.98 to 5.06.

Sera tested at day 17(a day of and just before primary vaccination) showed low mean titre of 291 ± 66.226 and ranged from 63 to 443. S/P ration was 0.552 ± 3.153 E - 20 and 2 tested samples was reacted negatively, 2 samples was considered as suspicious and 1 sample was reacted positive. CV% was high (51%). Sera collected 15 days post booster dose of vaccination (day 39) showed that vaccination using drinking water route induced mean titre of 5353 ± 1384.69 and ranged from 2535 to 9018. The mean S/P ratio for this group was 2.126 ± 0.525 and all sera samples were reacted positively (Table 2). This result suggested that vaccination via drinking water (oral) route is superior and preferable to other routes of vaccine administration, followed by group C and A. The least was group D.

The present study showed that no significant increase in anti-IBDV antibody titre in all groups compared to the titre at day 17 (before vaccination) except group B. The results also showed that all groups including group B antibody titre level did not succeed to raise the titre level to its level of day one (MDA). No significant difference in titre levels between other groups. Coefficient of variation % (CV%) was high for all groups including group B and exceeding the cut off point (20 - 50%).

All groups showed post mortem gross lesions and graded from 2.67 in group B to 3.28 in group C compared to 3.63 in the control (group E). No group passed the challenge without showing clinical signs. Mortality rates varied greatly and was significantly ($P < 0.05$) lower in group B (12%) than in all groups, the highest mortality rate was recorded in group A and group E (the control) (32%) each.

Bursa to body weight x 10^{-3} ratio and histopathologic changes grades are recorded in Table 3 and the highest was in group D..

As shown in Table 1, the MDA became very low at day 17 (291), this result agreed with finding of Zaheer *et al.* (2003) who had reported MDA as minimal and its protective efficacy diminished after one week of age. No obvious explanation for the higher titre of anti body in group B compared to other groups. Group B seem to be better protected than other groups. This result could possibly indicate superiority of vaccination via drinking water compared to other routes.

The noted high CV% in this study may be explained by that birds within each group may have been exposed to water containing anti-IBDV vaccine at different degrees. Another suggestion is that birds in all groups were already have variable levels of anti - IBDV antibodies and vaccinations conserve this variation.

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